The ionic dependence of the histamine-induced depolarization of vasopressin neurones in the rat supraoptic nucleus

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1. The ionic basis of the histamine-induced depolarization of immunohistochemically identified neurones in the supraoptic nucleus (SON) was investigated in the hypothalamo-neurohypophysial explant of male rats. Histamine (0.1–100 μM) caused an H_1 receptor-mediated, dose-dependent depolarization of fifty of sixty-two vasopressin neurones in the SON. In contrast, twenty-three oxytocin neurones were either depolarized ($n = 6$), hyper-polarized ($n = 4$), or unaffected ($n = 13$) by histamine. Due to the low percentage of responding cells, oxytocin neurones were not further investigated.

2. Chelation of intracellular Ca^{2+} with 1,2-bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA; 100–500 mM) blocked the depolarization, whereas blocking Ca^{2+} influx and synaptic transmission with equimolar Co^{2+} or elevated (5–20 mM) Mg^{2+} in nominally Ca^{2+}-free solutions was without effect.

3. The amplitude of the histamine-induced depolarization was relatively independent of membrane potential. The input resistance was unaltered by histamine in nine neurones, but in nine other neurones it was decreased and in two neurones it was increased by more than 5%. Neither elevating extracellular K^+ nor addition of the K^+ channel blockers, apamin, d-tubocurarine, tetraethylammonium (TEA), or intracellular Cs^+ decreased the histamine effect. Indeed, broadly blocking K^+ currents with TEA and Cs^+ significantly increased the depolarization to histamine.

4. Tetrodotoxin (2–3 μM) did not inhibit the histamine-induced depolarization. However, equimolar replacement of $\sim 50\%$ of extracellular Na^+ with $Tris^+$ or N-methyl-D-glucamine reduced or eliminated the response.

5. The depolarization of vasopressin neurones by histamine thus requires extracellular Na^+ and intracellular Ca^{2+}. Activation of a Ca^{2+}-activated non-specific cation current or a Ca^{2+}–Na^+ pump are possible mechanisms for this effect.

The rat hypothalamic supraoptic nucleus (SON) contains neurones that produce vasopressin (VP) and oxytocin (OT), hormones that are released from nerve terminals in the neurohypophysis into the systemic circulation. Several lines of evidence suggest that histamine plays an important role in controlling the excitability of SON neurones. As demonstrated by immunohistochemical (Watanabe *et al.* 1984), anatomical (Weiss, Yang & Hatton, 1989), and electrophysiological (Weiss *et al.* 1989) data, a bilateral histaminergic projection from the hypothalamic tubero-mammillary nucleus impinges on neurones in the SON. Histamine H_1 receptors have also been localized in the SON using receptor autoradiography (Palacios, Wamsley & Kuhar, 1981).

There is substantial evidence indicating that histamine influences VP neurone activity and consequent hormone release. Central administration of histamine causes anti-diuresis and increases plasma VP levels in a variety of mammals (e.g. Bennett & Pert, 1974; Dogterom, Van Wimersma Greidanus & De Wied, 1976), and increases c-*fos* expression in VP- and OT-containing SON neurones (Kjær, Larsen, Knigge, Møller & Warberg, 1994). During periods of dehydration, inhibition of histamine synthesis suppresses VP secretion, implying that the central release of histamine may be important to VP release to this stimulus (Kjær, Larsen, Knigge & Warberg, 1995).

Electrophysiologically, histamine application typically excites SON neurones *in vivo* (Haas, Wolf & Nussbaumer, 1975)

and *in vitro* (Armstrong & Sladek, 1985; Yang & Hatton, 1989; Smith & Armstrong, 1993; Li & Hatton, 1996), although some cells are inhibited or exhibit no response. Electrical stimulation of the tuberomammillary nucleus also causes phasically firing SON neurones to become more excitable (Yang & Hatton, 1989). The majority of VP-immunopositive neurones in the SON are excited by histamine via a direct, H_1 receptor-mediated increase in the amplitude of the depolarizing after-potential (DAP) that follows current-evoked trains of action potentials, and by a slow membrane depolarization (Smith & Armstrong, 1993).

Recent data suggest that histamine reduces a K^+ leak conductance when intracellular Ca^{2+} is strongly buffered (Li & Hatton, 1996). However, little is known about the mechanisms by which the effects of histamine on VP neurones are produced with normal intracellular Ca^{2+}. In the present study, the ionic basis for the observed histamine-induced depolarization of immunohistochemically identified VP neurones in the SON was investigated using conventional intracellular recordings in the hypothalamoneurohypophysial explant. Our results implicate a Ca^{2+}- and Na^+-dependent mechanism in the depolarizing response of VP neurones to histamine.

METHODS

Explant preparation

In vitro intracellular recordings were conducted in SON magnocellular neurones in the hypothalamo-neurohypophysial explant prepared from male Sprague–Dawley rats (Sasco, Wilmington, MA, USA) according to previously defined protocols (Armstrong, Gallagher & Sladek, 1985; Armstrong & Sladek, 1985). Briefly, animals weighing 125–250 g were anaesthetized with sodium pentobarbitone (100 mg kg^{-1} i.p.), and perfused intracardially with cold, oxygenated (95% O_2–5% CO_2) artificial cerebrospinal fluid (ACSF) consisting of (mM): 124 NaCl, 25 NaHCO$_3$, 3 KCl, 1·24 NaH$_2$PO$_4$, 10 glucose, 2·0 CaCl$_2$ and 1·3 MgSO$_4$; pH was 7·3–7·4 and the osmolality, 290–300 mosmol (kg H$_2$O)$^{-1}$. The brain was removed and the ventral surface of the hypothalamus was stripped of meninges. Together with the neurohypophysis, the ventral hypothalamus was excised with iris scissors and placed on a piece of filter paper on the perforated floor of a Plexiglass recording chamber. Warmed (32–33 °C), oxygenated ACSF flowed from a pipette positioned above the tissue.

Intracellular recording

Electrodes were pulled on a Flaming–Brown P-80/PC micropipette puller from 1·5 mm glass containing a microfilament and filled with 1·5–2% biocytin (Sigma) or Neurobiotin® (Vector Labs, Burlingame, CA, USA) in 1–2 M KCl, 1 M potassium methylsulphate, 2 M potassium acetate, or 2 M potassium acetate plus 0·1 M KCl. In some cases, neurones were impaled with electrodes containing 2 M CsCl or caesium acetate to block K^+ currents, or 100–500 mM 1,2-bis(2-aminophenoxy)ethane-*N,N,N′,N′*-tetra-acetic acid (BAPTA; Sigma) in 2 M potassium acetate to chelate intracellular Ca^{2+}. Electrode resistance ranged between 50 and 200 MΩ.

Following impalement, neurones were considered stable if the action potential amplitudes were at least +60 mV from threshold to peak for at least 10 min under normal recording conditions (i.e. no poisons or channel blockers). Continuous DC recordings made with a conventional active bridge amplifier were displayed on an oscilloscope and chart recorder, and digitized (Neurodata) at 44 kHz for storage on videotape. In addition, records were acquired at differing rates using the Labmaster TL-1 and an AT-style microcomputer running pCLAMP (Axon Instruments).

Input resistance was measured during histamine application by measuring voltage responses resulting from the injection of negative rectangular current pulses through the electrode. The effect of histamine on input resistance was determined either by measuring the linear slope resistance through resting membrane potential after readjusting the membrane to pre-histamine voltage with DC injection, or by comparing single non-rectifying pulse responses (i.e. within the linear current–voltage range) at the peak of the histamine response. The latter was done by either readjusting the membrane potential with DC injection during the depolarization or by comparing pulses at the peak depolarization with those evoked at the same membrane potential (controlled by DC injection) evoked prior to drug application.

In many cases the effects on membrane potential were examined while also evoking small groups of spikes in order to examine coincidental changes in DAP, some of which have been described previously (Smith & Armstrong, 1993). However, we have included the appropriate tests to determine whether the consequences of such activity (e.g. Na^+ and Ca^{2+} entry, K^+ efflux) influence the effects described, and these tests are relevant in determining the role of such ions when the neurone is depolarized to spike threshold.

Drug application

Histamine or its respective receptor agonists and antagonists were dissolved in ACSF and applied to the tissue either continuously or by bolus injection into the perifusion line. For the bolus injections of histamine and agonists, the drug concentrations given are approximations based on a 10% dilution factor of the injectate (Armstrong & Sladek, 1985). Histamine dihydrochloride (0·1–100 μM) was purchased from Sigma. Multiple applications to the same cell were made at a minimum of 10 min intervals to avoid possible false negative responses due to desensitization of histamine receptors themselves or of intracellular effector mechanisms associated with activation of those receptors. Receptor agonists and antagonists used were the H_1 receptor agonist, 2-thiazolylethylamine (Smith-Kline Beecham, Welwyn, Herts, UK; 1–100 μM), the H_2 receptor agonists, dimaprit or impromidine (Smith-Kline Beecham; 1–100 μM), the histamine H_1 receptor antagonist, promethazine (Sigma; 0·1–1 μM), and the H_2 receptor antagonist, cimetidine (Sigma; 0·1–50 μM). Apamin, *d*-tubocurarine (dTC), tetraethylammonium chloride (TEA), 4-aminopyridine (4-AP) and tetrodotoxin (TTX) were all purchased from Sigma. Solutions containing Co^+ or 4-AP were buffered with Tris–HCl instead of NaHCO$_3$ and contained no phosphate to avoid precipitation.

Statistical analysis

Regression analysis was used to determine the relationship of histamine-induced depolarizations to the membrane potential at which responses were evoked. The effects of histamine and other drug treatments were compared to pre-drug values using the non-parametric Wilcoxon paired sample test unless otherwise indicated.

Between-group comparisons were made with the non-parametric Mann–Whitney U test. Error terms given are standard errors of the mean.

Intracellular and immunohistochemical staining

Biocytin- or Neurobiotin-injected neurones were double labelled as described previously (Smith & Armstrong, 1993; Armstrong, Smith & Tian, 1994). Following each recording experiment, explants were placed in fixative consisting of 4% (w/v) paraformaldehyde and 0·2% (w/v) picric acid in 0·15 M NaPO$_4$ buffer (pH 7·3). After 1–2 days fixation at 4 °C, the explant was embedded in agar, and 50 μm vibratome sections were taken in the horizontal plane. Immunohistochemical identification of VP or OT neurones was performed in conjunction with histochemical localization of the injected biotin for a subset of the recorded neurones using semi-thin plastic sections. Briefly, for VP neurones, a rabbit antiserum to VP-neurophysin (VP-NP) was used (Roberts, Robinson, Fitzsimmons, Grant, Lee & Hoffman, 1993; supplied by Dr Alan G. Robinson, UCLA). For OT neurones, a mouse monoclonal antibody to OT-NP was used (Ben-Barak, Russel, Whitnall, Ozato & Gainer, 1985; supplied by Dr Harold Gainer, NIH). The neurone was first localized with avidin coupled either to Texas Red or 7-amino-4-methylcoumarin-3-acetic acid (AMCA). Adjacent 1–2 μm sections through the fluorescently labelled neurone were reacted separately for one of the two antibodies or, in some cases, the same section was co-incubated with both antibodies. The latter was possible because the primary antibodies were generated in different species. The immunochemical product was revealed either after standard peroxidase–anti-peroxidase methodology (adjacent sections) or by using a cocktail of fluorescently labelled secondary antibodies on a single section (fluorescein-conjugated goat anti-rabbit for VP-NP and rhodamine-conjugated goat anti-mouse IgG, or OT-NP). Fluorescent sections were viewed under epifluorescence using a Nikon Optiphot equipped with the following Nikon filters:

for Texas Red and rhodamine, G-2A; for fluorescein, B1-E; for AMCA, UV-2A.

RESULTS

General

All recordings were made from either the anterior or tuberal portion of the SON. The data presented are from sixty-two neurones found immunoreactive for VP-NP, and twenty-three neurones immunoreactive for OT-NP. Figure 1 shows an example of an identified neurone. An additional two neurones were immunopositive for both peptides and were not further considered in this analysis. For simplicity, neurones will be referred to as VP or OT neurones. Identified VP neurones had a resting membrane potential of -63 ± 2 mV, input resistance of 206 ± 10 MΩ, and action potential amplitude of 82 ± 2 mV. Identified OT cells had a resting membrane potential of -63 ± 3 mV, input resistance of 188 ± 12 MΩ, and action potential amplitude of 77 ± 2 mV.

Immunohistochemically identified OT neurones demonstrated a variable response to histamine, with six cells being depolarized and four cells being hyperpolarized. The depolarization of four of the six OT neurones occurred following injection of Cl$^-$ from KCl- or CsCl-filled electrodes. Application of histamine to the majority of OT neurones (13 of 23 cells) resulted in no change in membrane potential. The effects of histamine on OT neurones were variable and inconsistent. Because of this variability, and because most OT cells were not sensitive to histamine, no further
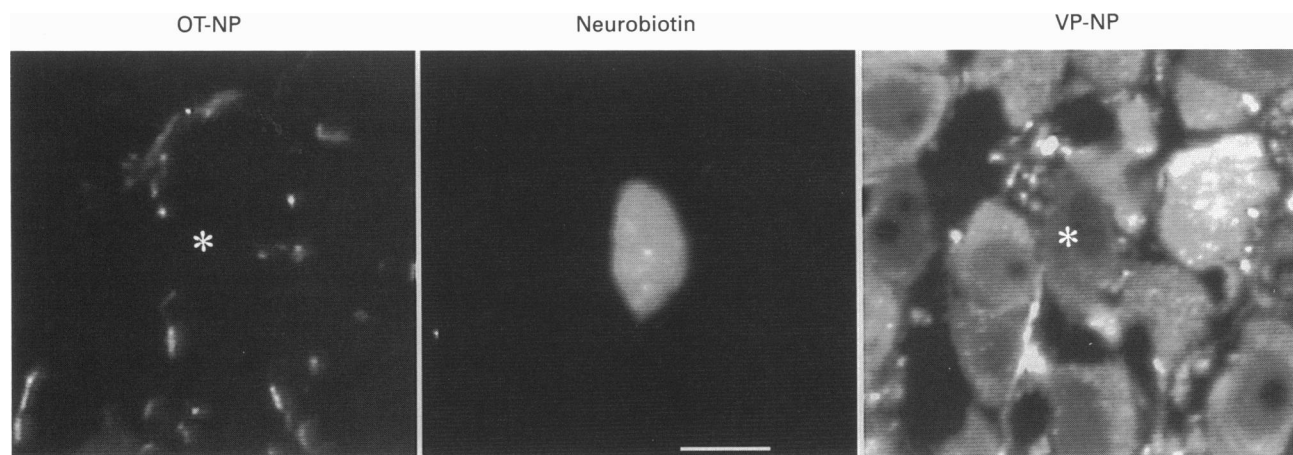

Figure 1. Immunohistochemical identification of a Neurobiotin-filled neurone

All three panels are photomicrographs of the same 2 μm plastic section which had been incubated with a cocktail of antibodies to oxytocin-neurophysin (OT-NP) and vasopressin-neurophysin (VP-NP). Middle panel, the neurone fluoresces with a UV filter locating the avidin–AMCA attached to the Neurobiotin marker. Left panel, the cell was negative for OT-NP (asterisk). A few OT-NP-positive fibres are visible. Right panel, the cell was positive for VP-NP (asterisk), as were most of the neurones in this region of the SON. Scale bar, 50 μm.

examination of the effects of histamine on OT cells was performed.

Effects of histamine on identified vasopressin neurones

Histamine was either bath applied or briefly injected into the perifusing line for 5 s. The bolus injections resulted in a transient application from which any effects could be readily washed out, minimizing potential desensitization. Drugs were considered to have had an effect if: (1) application resulted in a sustained depolarization of at least 2 mV; (2) the onset of the depolarization was consistent (within 1 min of introduction into the perfusion system; see Armstrong & Sladek, 1985); and (3) the effect was reversible. Response amplitudes were measured as the mean membrane potential over 10–20 s during peak depolarization. The predominant effect of histamine on the membrane potential of VP cells was a dose-dependent depolarization (50 of 62 cells) of 2 to 13 mV (Fig. 2). A 5 s bolus application into the perifusing line typically resulted in a 1–3 min response. Twelve VP cells exhibited no change in membrane potential in response to histamine and no VP neurones were hyperpolarized. As previously reported (Smith & Armstrong, 1993; Li & Hatton, 1996), the depolarization was mediated by H_1 but not H_2 receptors. The depolarization of VP neurones was mimicked by the H_1 receptor agonist, 2-thiazolylethylamine (1–100 μM; $n = 10$) and blocked by the H_1 receptor antagonist, promethazine (0·1–1 μM; $n = 5$), but unaffected by the H_2 antagonist cimetidine (1–50 μM; $n = 8$) and the H_2 agonists, dimaprit or impromidine (1–100 μM; $n = 5$).

Histamine had no reliable effect on input resistance when tested on twenty VP neurones ($P > 0.05$). However, the input resistance of individual cells could be increased (8 ± 1%; $n = 2$), decreased (12 ± 3%; $n = 9$) or unchanged
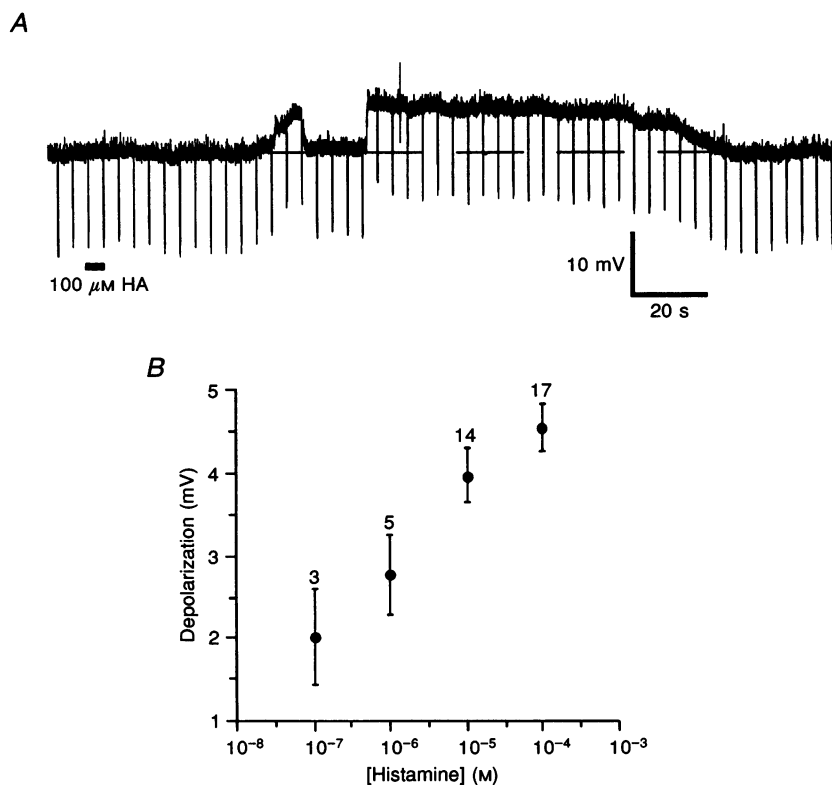

Figure 2. Depolarization of an immunohistochemically identified VP neurone by histamine

A, record showing the depolarizing effect of histamine (HA) on a VP neurone at resting membrane potential (−64 mV), indicated by the dashed line. The single spike near the middle of this record is clipped for presentation purposes. Hyperpolarizing rectangular current pulses (0·1 nA, 150 ms) were given every 4 s throughout the record to monitor changes in input resistance. In normal ACSF, a bolus application of 100 μM histamine caused the cell to depolarize ∼7 mV. The delay to the depolarization (∼45 s) is due to the delay in the perfusion line. Near the peak of the depolarization, the membrane was hyperpolarized to the pre-histamine membrane potential, revealing a histamine-induced decrease (∼10%) in input resistance in this neurone, which was not a consistent effect across neurones. *B*, dose dependence of the histamine-induced depolarization. Histamine was applied at various concentrations to 33 VP neurones. The number of trials for each dose is indicated above each point on the curve. Maximal responses were observed with 100 μM histamine. The minimum effective dose was 100 nM.

(<5%; n = 9). We further compared the input resistances of neurones of which the membrane had been hyperpolarized during histamine depolarization to the pre-drug value (e.g. Fig. 2; n = 12) with those of neurones depolarized before histamine application to a level equal to that during histamine application (n = 8), and found no difference between the two groups of neurones (P > 0·05).

Effects of synaptic blockade and Ca²⁺

Poisoning the explant with TTX (2–3 μM) failed to block the depolarization due to histamine (n = 7; Fig. 3) or the H_1 agonist, 2-thiazolylethylamine (n = 3). In addition, when both synaptic transmission and Ca^{2+} influx were reduced by eliminating extracellular Ca^{2+} and elevating extracellular Mg^{2+} (5·5–20 mM; n = 4) or by replacement of Ca^{2+} with equimolar Co^{2+} in the ACSF (n = 3), the histamine-induced depolarization remained (Figs 3 and 4). Input resistance was unchanged (<5%) by these solutions. These data confirm that histamine acts postsynaptically to depolarize VP cells and that the depolarization is not dependent upon a TTX-sensitive channel or the flow of Ca^{2+} into the cell.

Calcium chelation

The dependence of the excitatory effects of histamine on intracellular Ca^{2+} was determined by testing neurones recorded with electrodes containing the Ca^{2+} chelator BAPTA (100–500 mM), the results of which are shown in

Fig. 5. BAPTA entry into the cell was determined by monitoring changes in spike width as well as the decrease in amplitude of the Ca^{2+}-dependent after-hyperpolarization (AHP) following evoked bursts of spikes (Kirkpatrick & Bourque, 1991). Depending on the concentration in the electrode and the amount of negative current applied, BAPTA took from 2–3 min using the highest concentrations to 15–20 min with the lowest concentrations in the electrode to fully exert its effects on the AHP. No depolarizations occurred following histamine application in two VP neurones recorded with electrodes containing 300–500 mM BAPTA. In three neurones recorded with 100–200 mM BAPTA, a depolarization could be elicited by histamine when applied within 15 min of impalement. Following injection of BAPTA with combined negative DC current and negative rectangular current pulses for 15–20 min, histamine failed to elicit a depolarization in any of the three VP neurones that depolarized prior to BAPTA injection. BAPTA injection had little effect on the input resistance of these three cells (157 ± 23 vs. 153 ± 26 MΩ). Some concentration of intracellular Ca^{2+} is therefore required for histamine to depolarize VP neurones.

Effects of varying membrane potential

To examine the effect of changing membrane potential on the histamine-induced depolarization, neurones were held at various membrane potentials with intracellular current
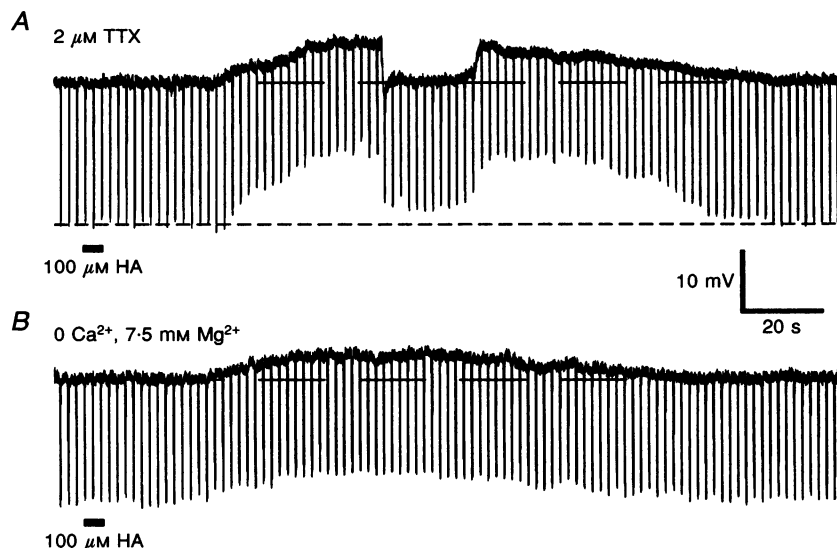

Figure 3. Histamine depolarized VP neurones in the absence of synaptic transmission

A, in 2 μM TTX, histamine depolarized this neurone by ∼7 mV. Hyperpolarizing pulses (220 ms, 0·1 nA) were given throughout the record to monitor input resistance. Near the peak of the depolarization, the membrane was hyperpolarized to the pre-histamine membrane potential, revealing a histamine-induced decrease (∼10%) in input resistance. Short dashed line indicates membrane potential reached by the hyperpolarizing pulses used to measure input resistance before histamine-induced depolarization. *B*, nominally Ca^{2+}-free solutions containing elevated Mg^{2+} also failed to inhibit the depolarizing response to histamine. Membrane potential was −68 mV. Hyperpolarizing pulses (220 ms, 0·1 nA) were applied throughout the record. No change in input resistance (<5%) was associated with the depolarization in this neurone. Long dashed lines indicate resting membrane potentials of −57 mV (*A*) and −68 mV (*B*).

injection during histamine application. Histamine depolarized VP cells over a wide range (−100 to −48 mV) of membrane potentials (Fig. 6), even within the same neurone, and there was no correlation between the amplitude of the depolarization and membrane potential.

Chloride

We previously reported that the histamine-induced enhancement of the DAP did not depend on intracellular Cl⁻ concentration (Smith & Armstrong, 1993). Maximal doses of histamine (100 μM), determined by the dose–response
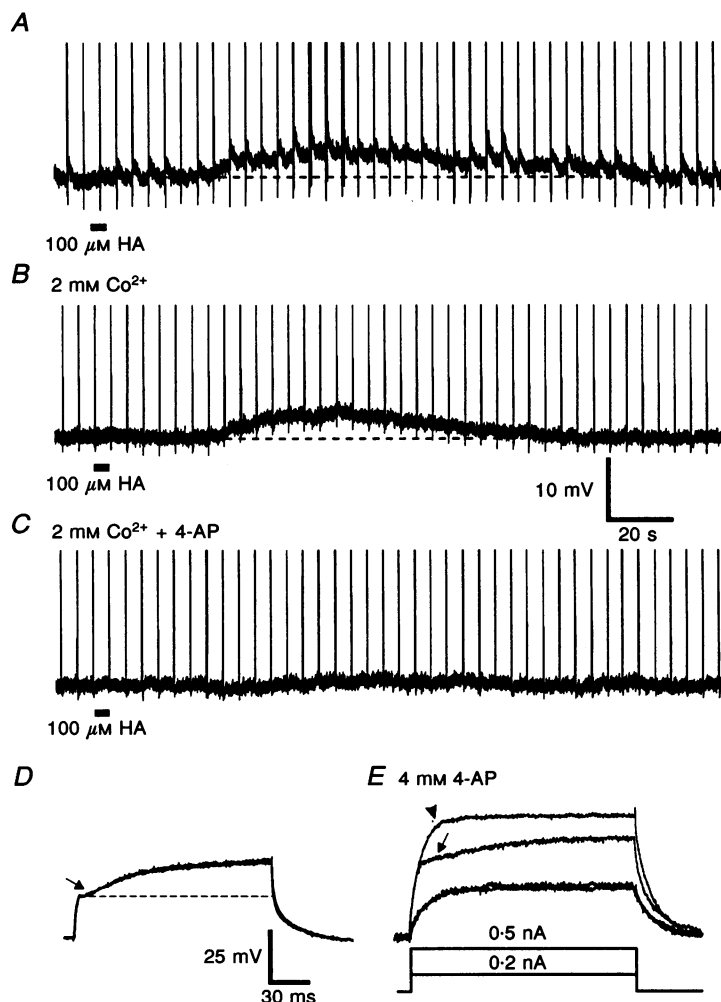

Figure 4. The histamine-induced depolarization persisted when Ca^{2+} currents were blocked but was abolished by 4-AP

All records are from the same neurone. *A*, the administration of histamine to a cell in standard ACSF results in a 4–5 mV depolarization. Rectangular depolarizing current pulses (0·05 nA) were given throughout the record to elicit spikes. Input resistance was 212 MΩ. *B*, equimolar replacement of extracellular Ca^{2+} with Co^{2+} resulted in the complete abolition of the DAP and reduction in the slow AHP following elicited spikes. The Ca^{2+}-independent hyperpolarizing after-potential following each spike in the train remains evident (downward deflections). Histamine applied during Ca^{2+} current blockade resulted in a depolarization of similar amplitude to that observed in standard ACSF. *C*, the addition of 4 mM 4-AP to the ACSF resulted in the reduction of the hyperpolarizing after-potentials following evoked spikes, and blocked the depolarizing effect of histamine. Histamine was applied at the same holding potential (−63 mV) in *A–C*. *D*, the presence of the transient outward rectifier (I_{to}) (Bourque, 1988) is demonstrated in a VP neurone by applying a depolarizing current pulse of 0·6 nA from a holding potential of −87 mV. Note the strong, transient outward rectification seen at the beginning of the trace (arrow). A similar pulse from the same membrane potential in the presence of histamine (overlapping trace) indicates that histamine is not strongly influencing I_{to} in VP cells. *E*, conversely, application of 4-AP to a different cell in the presence of 3 μM TTX abolished the outward rectification, presumably by reducing I_{to}. Arrow, trace obtained in TTX only; arrowhead, trace obtained in the presence of TTX plus 4-AP. Membrane potential was −84 mV.

characteristics for the histamine-induced depolarization, resulted in similar depolarizations, with Cl^--loaded cells ($n = 6$) depolarizing $4\cdot6 \pm 0\cdot5$ mV in nine applications, and non-Cl^--loaded cells ($n = 9$) depolarizing $4\cdot5 \pm 0\cdot1$ mV in thirteen applications ($P > 0\cdot05$). The excitatory effects of histamine on VP cells were therefore not mediated by a change in Cl^- conductance.

Potassium

The K^+ dependence of the effects of histamine was determined pharmacologically and by ion substitution. Apamin (50–100 nM) or dTC (100–200 μM), both of which block the slow AHP following evoked trains of action potentials (Bourque & Brown, 1987), failed to affect the histamine-induced depolarization in nine VP cells tested (Smith & Armstrong, 1993). These data imply that the direct depolarization by histamine is not due to a reduction in the Ca^{2+}-dependent K^+ current underlying the slow AHP in VP cells.

Neurones impaled with 2 M CsCl or caesium acetate in the electrode rapidly depolarized and exhibited broad action potentials. It was thus necessary to test histamine when holding the cell membrane potential to < -60 mV with negative current. The input resistance of Cs^+-loaded neurones averaged 215 ± 3 MΩ ($n = 6$), which is similar to that of neurones recorded with K^+-filled electrodes (see above). Histamine (100 μM) induced a depolarization ($9\cdot3 \pm 1\cdot7$ mV) in six of six Cs^+-loaded VP cells (Fig. 7) that was larger than that observed in non-Cs^+-loaded cells ($4\cdot5 \pm 0\cdot5$ mV). Further addition of TEA (7–20 mM) to two of these neurones also failed to block or diminish the depolarization. Bathing neurones in 7–20 mM TEA with K^+-filled electrodes also broadened evoked action potentials and failed to block or inhibit the histamine-induced depolarization in six of six VP neurones. Indeed, the depolarization to histamine was significantly larger in these neurones after TEA was applied ($5\cdot2 \pm 1\cdot9$ mV before *vs.* $8\cdot0 \pm 1\cdot4$ mV after TEA; $P < 0\cdot05$). TEA significantly
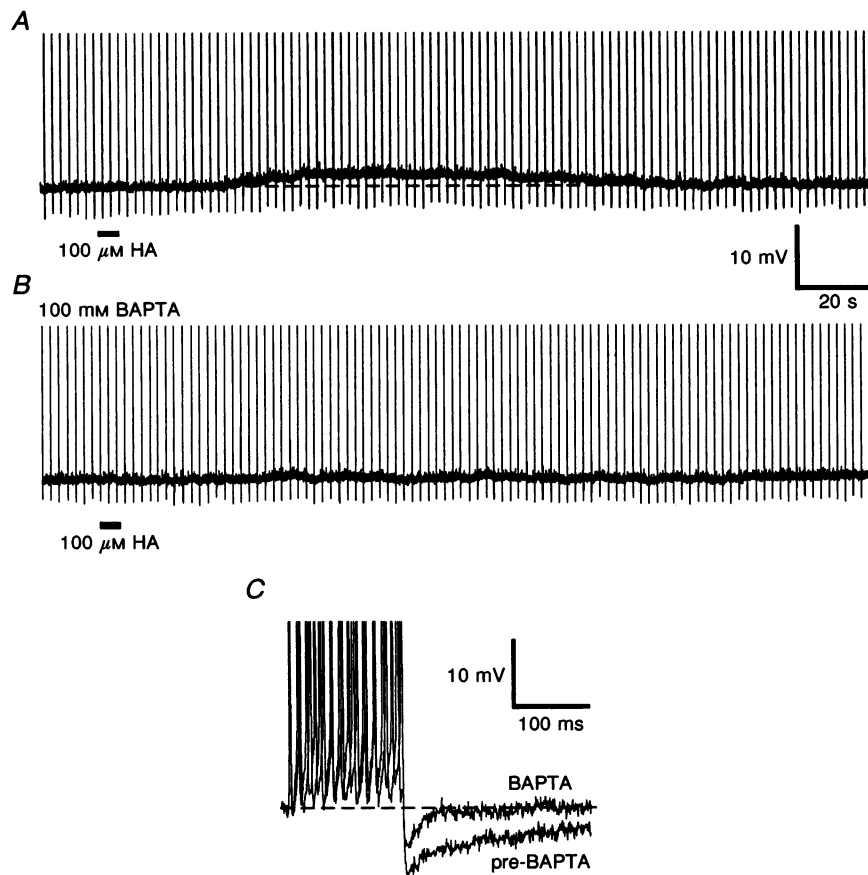

Figure 5. Chelation of intracellular Ca^{2+} with BAPTA blocked the histamine-induced depolarization

A, this VP neurone responded to histamine when the amine was applied within 10 min of obtaining the recording using an electrode containing 100 mM BAPTA. *B*, following 15 min of negative current injection to eject BAPTA from the electrode, histamine had no effect on the membrane potential. Membrane potential (dashed line) was −60 mV for both applications. *C*, in the same cell, BAPTA injection decreased the amplitude and duration of the AHP following evoked bursts of spikes as shown with overlapping traces taken at the same membrane potential before and after injection.
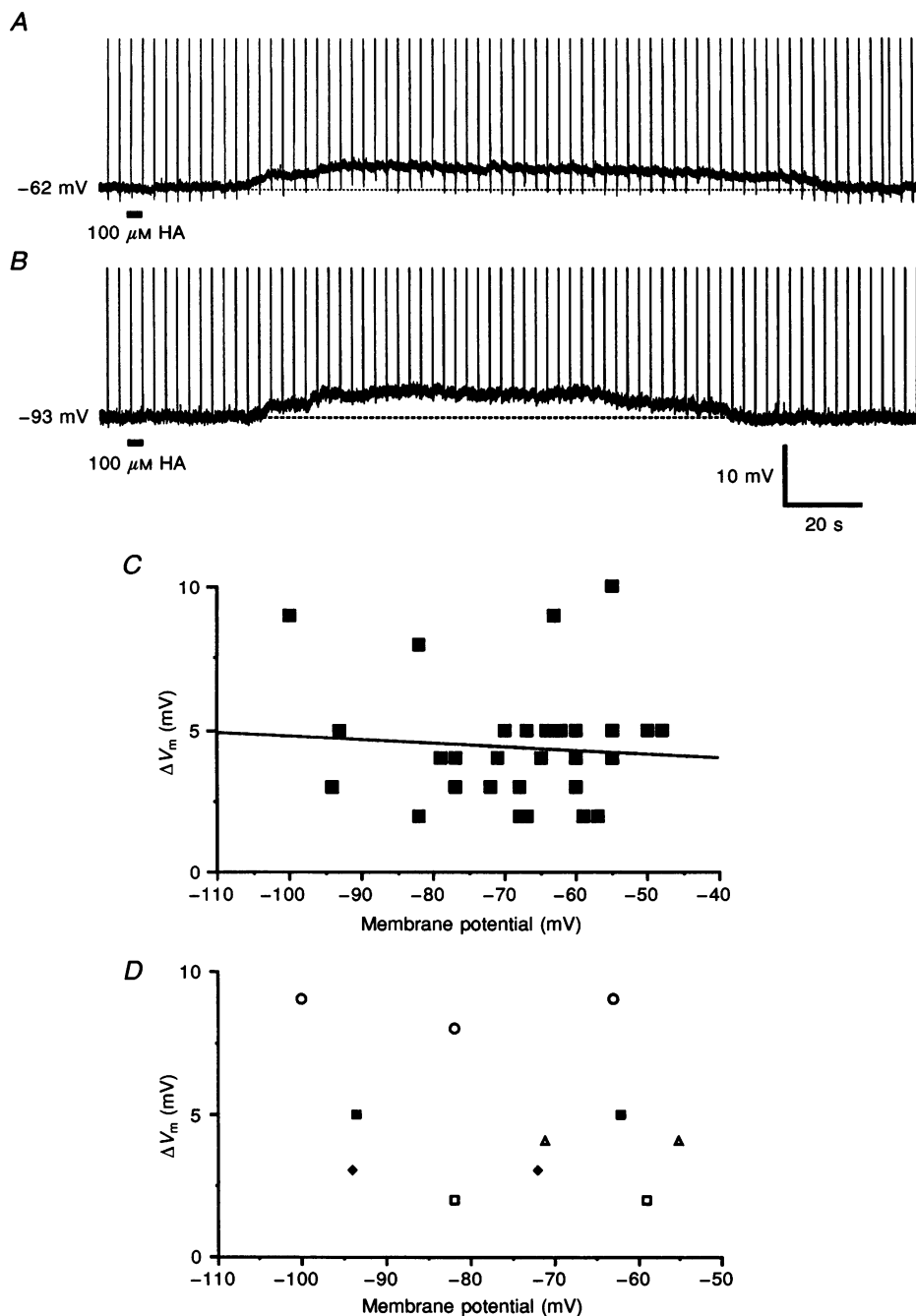
Figure 6. The histamine-induced depolarization is not strongly influenced by membrane potential

A, histamine depolarized this neurone ~5 mV from its resting membrane potential. Input resistance for this neurone was 160 MΩ. *B*, in the same neurone, histamine induced a similar depolarization when holding the membrane potential with negative DC current at −31 mV from rest. Depolarizing trains of action potentials were applied throughout both records; input resistance at this potential was 167 MΩ. *C*, application of 100 μm histamine to 19 VP cells, including those tested in the presence of TTX, resulted in a depolarization from a wide range of membrane potentials (−48 to −100 mV). The regression line through the graph indicates the lack of tight correlation ($r^2 = 0.006$) between the amount of depolarization and the membrane potential of the cell. *D*, in five VP neurones, 100 μm histamine induced a similarly sized depolarization when given at 2 or 3 different potentials separated by 15–37 mV in each cell. Each type of symbol represents a different neurone.

Figure 7. Effects of blocking repolarizing K⁺ currents with Cs⁺ and TEA

A, application of histamine to a cell that was injected with 2 M caesium acetate resulted in a depolarization of about 10 mV. Single spikes were elicited with 3 ms depolarizing (0·5 nA) current pulses throughout the chart record to monitor spike width. Membrane potential (−75 mV) was maintained with constant current (−0·12 nA) injection. Input resistance for this neurone was 228 MΩ. *B*, after bathing the same cell in 7 mM TEA, histamine produced a similarly large depolarization. Membrane potential was held at −72 mV (−0·15 nA DC); input resistance was 247 MΩ.
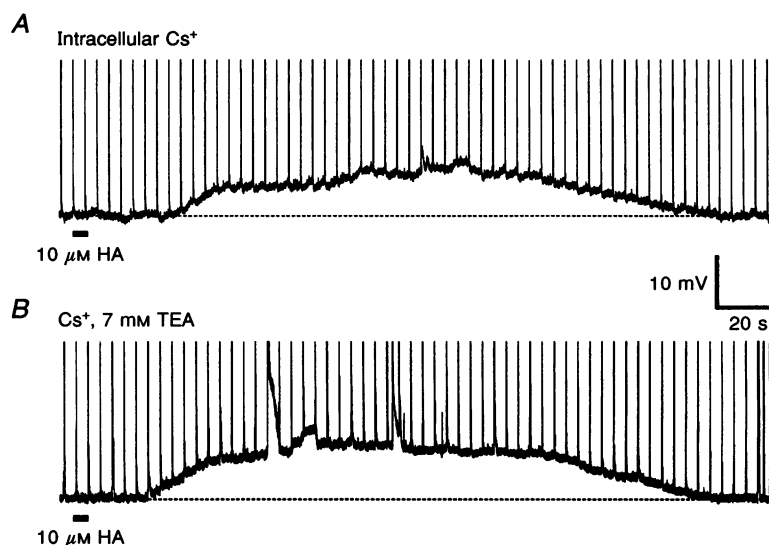
increased the input resistance of these neurones from 189 ± 10 to 257 ± 15 MΩ (*P* < 0·05), which could explain the increased response to histamine (i.e. the histamine-induced current was probably not enhanced by TEA). It is more difficult to evaluate the larger response with internal Cs⁺ blockade with regard to input resistance, because Cs⁺ appears to flow quickly into the cells after impalement, as judged by the immediate increased spike width and necessity of holding the membrane potential of the neurone with negative current.

The depolarizing effect of histamine on VP neurones was completely blocked by bathing the explant in 4–5 mM 4-AP in five of five cells tested (Fig. 4). In two cases this blocking effect was partially reversed by washing the explant in 4-AP-free ACSF for about 1 h. These data suggest that the effects of histamine might be due to a reduction in 4-AP-

sensitive K⁺ current. However, the transient outward rectification observed during depolarizing pulses from hyperpolarized membrane potentials, which is also suppressed by millimolar concentrations of 4-AP, was not affected by histamine under standard recording conditions (Fig. 4). The mean input resistance of these five neurones was not significantly altered by 4-AP (185 ± 30 vs. 174 ± 23 MΩ), although in two neurones 4-AP reduced the input resistance by ~15%.

The K⁺ dependence of the histamine-induced depolarization was assessed further by raising extracellular K⁺ from 3 to 9 mM (*n* = 6), using equimolar substitution of Na⁺ to retain osmotic balance. Elevation of extracellular K⁺ did not affect the depolarization induced by histamine in any of six VP neurones when tested at the same membrane potential as that prior to elevating K⁺ (Fig. 8). Although there was a

Figure 8. The depolarization was not strongly dependent on extracellular K⁺ concentration

A, at resting membrane potential (−57 mV), histamine produced an ~4 mV depolarization and increased spontaneous spike activity in standard ACSF (3 mM K⁺). Input resistance for this neurone was 200 MΩ. *B*, when extracellular K⁺ concentration was raised to 9 mM, histamine application at the same potential (controlled with constant current injection) resulted in a depolarization and increase in spiking similar to that observed in standard ACSF. Input resistance in elevated K⁺ was 165 MΩ.
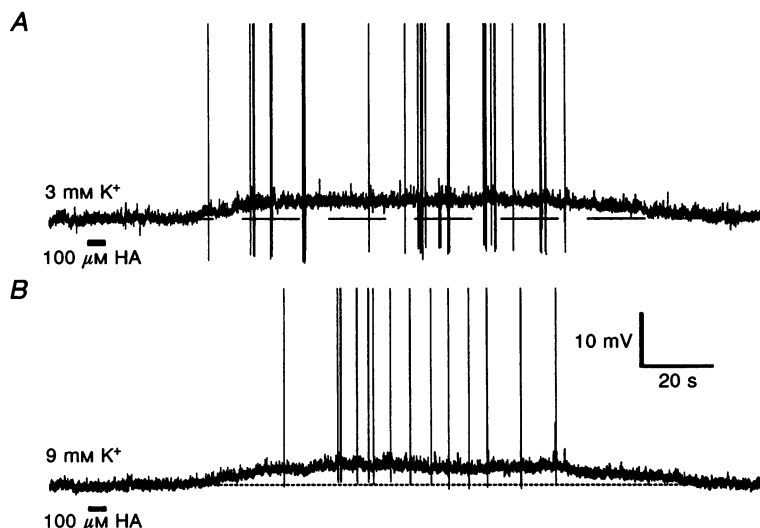
tendency for a decreased input resistance in response to high K^+ in some of these neurones (4 of 6 neurones showed decreases of 15–33%), the mean input resistance was not significantly altered (185 ± 19 vs. 161 ± 19 MΩ). Nevertheless, the fact that the depolarization was also similar in neurones in which input resistance was decreased would suggest that in these cases, a slightly larger current would have been necessary to evoke the same depolarization under high K^+ (on average, 21 vs. 27 pA).

Sodium

In addition to examining the dependence on TTX-sensitive channels (see above), the role of Na^+ was examined further by replacing 70–75 mM extracellular NaCl with equimolar concentrations of Tris–HCl ($n = 2$) or N-methyl-D-glucamine ($n = 2$). In all four VP neurones the histamine-induced depolarization was strongly reduced or eliminated when tested at the same membrane potential (Fig. 9). In general, Na^+-replacement studies were difficult because many neurones were lost in the exchange (usually when returning to normal media) despite being careful to ensure that pH and osmotic pressure were identical in the normal and Na^+-reduced media. This was especially true with attempts made at large replacements (120 mM). In the one additional neurone which tolerated this larger change, the histamine-induced depolarization was completely blocked. These data suggest that a non-TTX-sensitive Na^+-dependent mechanism may be involved in the response of VP neurones to histamine. Replacement of Na^+ did not consistently affect the input resistance of any of these neurones.

DISCUSSION

The predominant effect of histamine on most identified VP neurones in this study was an H_1 receptor-mediated, dose-dependent slow and small depolarization of the membrane potential. The ability of histamine to depolarize VP cells and bring the membrane potential close to or above spike threshold would enhance the ability of the cell to fire bursts of spikes in response to small depolarizing stimuli which might not otherwise cause the neurone to fire. For example, from a mean resting membrane potential of ~ -63 mV, a typically sized (~ 5 mV) DAP would probably not be sufficient to induce phasic bursts in most VP neurones. Excitatory synaptic inputs, even large inputs that cross spike threshold, would therefore not necessarily lead to phasic bursting. However, when coupled with a small depolarization, synaptic potentials could more readily evoke spikes and a DAP. Since the DAP is also larger at more depolarized potentials, the histamine-induced depolarization may increase the effectiveness of relatively small synaptic inputs by both allowing them to generate spikes and by further increasing the likelihood that those spikes will cause a DAP that crosses spike threshold. The concurrent H_1 receptor-induced increase in DAP amplitude (Smith & Armstrong, 1993) would further amplify this effect, allowing histamine to profoundly influence the firing pattern of the neurone. In contrast, histamine inconsistently and variably affected the membrane potential of OT neurones (Smith & Armstrong, 1993). A more comprehensive analysis of some interesting inhibitory effects of histamine-mediated synaptic actions on OT neurones has been reported (Yang & Hatton, 1994).
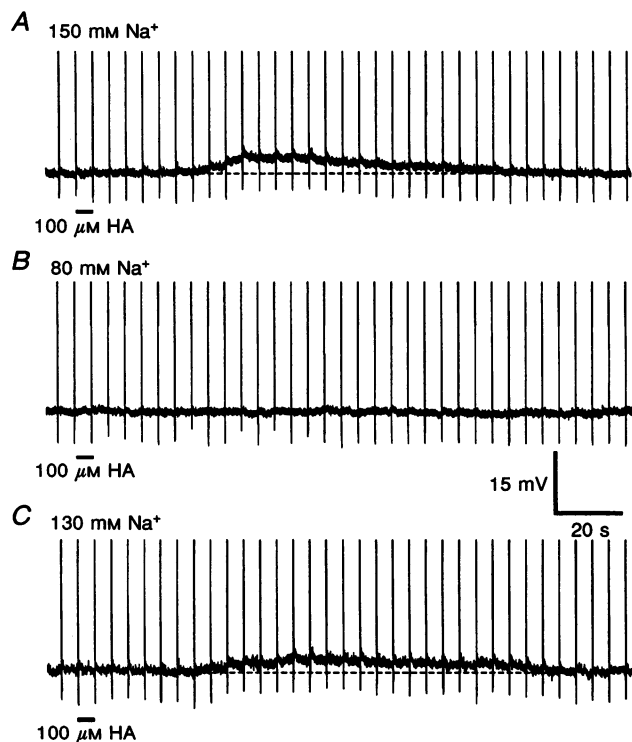

Figure 9. The histamine-induced depolarization is Na^+ dependent

A, application of histamine at resting membrane potential (-62 mV) elicited an ~ 5 mV depolarization. B, equimolar substitution of $Tris^+$ for 70 mM Na^+ prevented the effect of histamine. C, histamine again depolarized the neurone following reinstatement of 50 mM Na^+ to the ACSF in Tris-buffered medium. Depolarizing spike trains (0·5 nA, 50 ms, 50 Hz) are being evoked at 10 s intervals throughout the records.

Direct depolarization

The depolarizing effect of histamine on VP neurones occurred when spike-dependent synaptic activity was blocked by TTX or when Ca^{2+} currents were blocked, indicating that histamine directly depolarizes VP cells. Although not pharmacologically isolated in this study, the H_3 receptor-mediated release of histamine or other neurotransmitters from presumptive presynaptic terminals contacting VP neurones depends on Ca^{2+} influx at the terminals (Arrang, Garbarg & Schwartz, 1983) and therefore was probably blocked with Ca^{2+} current blockade. Although histamine may still have unappreciated presynaptic or intercellular actions, the present and previous results (Armstrong & Sladek, 1985; Yang & Hatton, 1989; Smith & Armstrong, 1993; Li & Hatton, 1996) indicate that histamine directly depolarizes VP neurones by acting at postsynaptic H_1 but not H_2 receptors.

Calcium

A variety of H_1 receptor-mediated events have been shown to be Ca^{2+} dependent (Schwartz *et al.* 1982), suggesting that the excitation of VP neurones by histamine could involve Ca^{2+}. Second messenger actions were not investigated in the present studies. However, several lines of evidence suggest that H_1 receptor activation can activate the inositol phosphate second messenger cascade (Daum, Downs & Young, 1984) and release Ca^{2+} from intracellular stores in a variety of cell types. For example, in rat trigeminal ganglion neurones, histamine can transiently increase intracellular Ca^{2+} concentration by nearly 3000 % (Tani, Shiosaka, Sato, Ishikawa & Tohyama, 1990). In contrast, the depolarization of VP neurones by histamine did not require the presence of extracellular Ca^{2+}, and therefore was not due primarily to a change in Ca^{2+} current. This is noteworthy because histamine was often applied while evoking spikes with intracellular current injection to examine the DAP (Smith & Armstrong, 1993). Neither the Ca^{2+} entry nor the DAP subsequent to such activity was necessary for the histamine-induced depolarization (see Figs 3 and 4). When spikes were elicited, individual bursts were separated by at least 5 s, allowing any evoked DAP to decay completely before the next burst was elicited. Thus, even when the depolarizing effect of histamine was examined when evoking a DAP, it was not due to summation of Ca^{2+}-dependent DAPs.

Recently, Li & Hatton (1996) described a Ca^{2+}-independent, histamine-induced depolarization using whole-cell recordings. In agreement with their study, we observed the effect of histamine to be independent of synaptic transmission and Ca^{2+} current blockade. However, in our experiments, the depolarization was blocked when intracellular Ca^{2+} was chelated, indicating that some concentration of intracellular Ca^{2+} is required for the depolarization to occur. We used 100–500 mM BAPTA (concentration in a sharp electrode), an order of magnitude greater than that used by Li & Hatton in patch pipettes (1996), and were able to obtain depolarizations prior to, but not following, ejection of BAPTA. A difference in the final intracellular BAPTA and Ca^{2+} concentrations achieved by these two methods, especially remotely in the dendritic arbor, is one possible explanation for the disparate results of these two studies.

Chloride

Histamine has been demonstrated to alter a Cl^- conductance in lobster olfactory ganglion cells (McClintock & Ache, 1989) and evidence also exists for a possible H_2 receptor-mediated increase in a Cl^- conductance in OT neurones (Yang & Hatton, 1994). Injection of Cl^- did not affect the amplitude or direction of the histamine-induced change in membrane potential of identified VP cells. Therefore, as with the increase in DAP amplitude (Smith & Armstrong, 1993), the histamine-induced depolarization is Cl^- independent.

Potassium

In hippocampal CA1 pyramidal neurones, histamine acts via H_2 receptors to block a Ca^{2+}-activated K^+ conductance, reducing the AHP following an evoked burst of spikes and the spike frequency adaptation during such bursts, effectively maintaining the cell in a more depolarized state (Haas & Green, 1986). Histamine depolarized VP cells in the presence of apamin and dTC , both of which block the Ca^{2+}-dependent K^+ current underlying the AHP in SON neurones (Bourque & Brown, 1987). This indicates that a reduction by histamine in the current underlying the Ca^{2+}-dependent AHP is not a primary factor in the histamine-induced depolarization. Similarly, the enhancement of the DAP is not dependent upon some change in the AHP (Smith & Armstrong, 1993). Finally, the fact that similarly sized depolarizations were obtained after blocking the AHP also suggests that activation of this current by histamine (which could have occurred by the putative increase in intracellular Ca^{2+}) did not oppose the depolarization.

Activation of α_1-adrenoreceptors in the SON results in a small depolarization (Randle, Bourque & Renaud, 1986), reportedly involving the reduction of a transient K^+ current (I_{to}) suggested to be active as a window current at potentials near rest (Bourque, 1988). Recent evidence also suggests that histamine can reduce a K^+ leak conductance (I_{KL}) in SON neurones using whole-cell recordings (Li & Hatton, 1996), and in thalamic neurones with sharp electrodes (McCormick & Williamson, 1991). However, the histamine-induced depolarization we observed was not primarily mediated by the reduction of a K^+ current. Firstly, the depolarization was not decreased by strong hyperpolarization towards the K^+ equilibrium potential. Secondly, the depolarization was not decreased by raising the K^+ equilibrium potential. Indeed, input resistance was decreased in some neurones when K^+ was elevated, suggesting that a larger current flow would have been necessary to produce the same depolarization. Finally, the depolarization was not attenuated but typically increased by extracellular TEA and/or intracellular Cs^+, treatments which block many K^+ currents, including those contributing

to membrane potential. The increased amplitude of the depolarization in neurones in which K$^+$ channels were blocked with TEA and Cs$^+$ probably reflects either inhibition of outward currents that might oppose the histamine-induced depolarization, or more simply a larger voltage drop across a membrane of higher resistance.

Histamine-induced depolarizations have been associated with an increased input resistance in sharp electrode studies of thalamic neurones (McCormick & Williamson, 1991), and in SON neurones with whole-cell recordings, but only when intracellular Ca^{2+} was buffered with BAPTA (Li & Hatton, 1996). Without Ca^{2+} chelation, Li & Hatton (1996) reported no change in membrane resistance, as in the present investigation. Taken together, these data suggest that histamine is altering the transmembrane flow of more than one ion type and probably affecting more than one channel. As a caveat, the dendrites significantly contribute to the electrotonic behaviour of many SON neurones (Armstrong & Smith, 1990) and therefore the membrane potential is not controlled isopotentially by somatic current injection. Thus, while the present study does not support the reduction of a K$^+$ leak current as the primary means of the depolarization, it remains possible that histamine alters a remotely (electrotonically) located or small-conductance K$^+$ current that is difficult to detect under normal conditions, even with whole-cell recordings (Li & Hatton, 1996). Such a conductance might be amplified when intracellular Ca^{2+} is buffered (e.g. by reducing opposing Ca^{2+}-dependent conductances), allowing it to be detected by whole-cell recordings.

In the light of the experiments indicating a negligible primary involvement of K$^+$ in the effect of histamine, the blockade of the depolarization with the K$^+$ channel blocker 4-AP appears anomalous. Although 4-AP blocks the voltage-dependent transient K$^+$ current, I_{to}, in SON cells (Bourque, 1988; Cobbett, Legendre & Mason, 1989), histamine had no effect on the outward rectification observed during depolarizing pulses from potentials negative to ~ −80 mV. Thus, even if I_{to} contributes to the resting potential in SON neurones as a window current (Bourque, 1988), the weight of the evidence suggests that histamine does not produce its depolarization by reducing this current. Of course, 4-AP may still be blocking the depolarization by interfering with a small or remote K$^+$ conductance which we have failed to unveil, as discussed earlier. In the study by Li & Hatton (1996), the effect of histamine on the K$^+$ leak current was blocked by a cocktail of 40 mM TEA, 20 mM 4-AP and 1–3 mM Cs$^+$, but these blockers were not tested independently or at smaller concentrations.

Alternatively, 4-AP has been demonstrated to alter intracellular Ca^{2+} concentration in a variety of preparations, including nerve terminals (e.g. Gibson & Manger, 1988). This may be due either to Ca^{2+} influx pre- and post-synaptically or to release from intracellular stores, as has been demonstrated in neurones (Gibson & Manger, 1988). Furthermore, 4-AP can act intracellularly to phosphorylate proteins and thus interfere with second messenger-

mediated events in synaptosomes (Heemskerk *et al.* 1991). The blockade of the histamine-induced depolarization by 4-AP may therefore be due to interference by the poison with the presumptive ability of histamine to alter intracellular Ca^{2+}, but this remains to be tested.

Sodium

In the present study, replacement of Na$^+$ with Tris or N-methyl-D-glucamine strongly reduced or eliminated the depolarizing effect of histamine. Among the possible consequences of a rise in intracellular Ca^{2+} concentration are activation of a non-specific cation current, which has been reported in the SON in response to dopamine (Yang, Bourque & Renaud, 1991), an activation of an electrogenic Ca^{2+}–Na$^+$ pump (Blaustein, 1988), or the activation of a Ca^{2+}-activated inward rectifier current (Schwindt, Spain & Crill, 1992). The dependence on intracellular Ca^{2+} and extracellular Na$^+$ of the histamine-induced responses supports all of these possibilities.

A small enhancement of the inward rectifying persistent Na$^+$ current (I_{NaP}; Stafstrom, Schwindt, Chubb & Crill, 1985) or I_h (Erickson, Ronnekleiv & Kelly, 1990; McCormick & Williamson, 1991) might depolarize the neurone. Both I_h and I_{NaP} identified in cat neocortical neurones are sensitive to internal Ca^{2+} chelation (Schwindt *et al.* 1992). The independence of the depolarization on extracellular K$^+$ concentration and membrane potential make it unlikely that histamine is affecting I_h, which is hyperpolarization-activated and carried by both Na$^+$ and K$^+$ (Erickson *et al.* 1990). Although there may be other sustained Na$^+$ currents which might vary in their TTX sensitivity, the I_{NaP} of Stafstrom *et al.* (1985) is TTX sensitive, unlike the histamine depolarization.

The H$_1$ receptor-induced depolarization shares similarities with the effects of dopamine D$_2$ receptor activation of unidentified SON neurones (Yang *et al.* 1991). Dopamine produced a slow, small, direct depolarization which required intracellular, but not extracellular Ca^{2+} and was sensitive to changes in extracellular Na$^+$. As with histamine, dopamine depolarized SON neurones similarly over a wide range of membrane potentials. Although the K$^+$ dependence was not assessed, the D$_2$ receptor-mediated depolarization of SON neurones was suggested to be due to a Ca^{2+}-dependent non-specific cation current (Yang *et al.* 1991). A non-specific cation (CAN) conductance that involves K$^+$ should be affected by altering the K$^+$ equilibrium potential, unless the permeability of the channel to K$^+$ is low relative to its permeability to Na$^+$, or the channels responsible are electrotonically remote from the recording site. Although there was a trend towards a decrease in input resistance (i.e. an increased conductance) in response to histamine, a large and consistent change in input resistance was not observed. However, the input resistance decrease associated with D$_2$ receptor activation was also small: in the order of 10–20% (Yang *et al.* 1991). Since input resistance was decreased when K$^+$ was elevated in four of six neurones, a

larger histamine-induced inward current would have been necessary to cause the equivalent depolarization. This could support the contention that histamine was acting through CAN channels since raising the K^+ equilibrium potential would reduce or eliminate outward K^+ movement at the potentials we examined (more negative than -55 mV in these experiments). However, increasing the driving force for Na^+ by membrane hyperpolarization should have increased the depolarization if this current were voltage independent, as it is in many cell types (Partridge & Swandulla, 1988). Thus, our data would be consistent only with a voltage- and calcium-dependent CAN conductance which is less active in the hyperpolarized range, similar to that described recently by Caeser, Brown, Gähwiler & Köpfel (1993). Although Tris is reported to pass through some CAN channels and N-methyl-D-glucamine is impermeant (Partridge & Swandulla, 1988), like Yang *et al.* (1991) we found that Tris also depressed the depolarization. This could still be consistent with some permeability to Tris, as the estimated reversal potential for the dopamine-induced depolarization is much more hyperpolarized with Tris substitution (Yang *et al.* 1991). Both Cs^+ and TEA permeate some CAN channels (Partridge & Swandulla, 1988), and the depolarization we achieved by filling neurones with Cs^+ would not support passage of this ion through the histamine-affected channel.

Another mechanism for the depolarization which is suggested by the sodium and calcium dependence is an electrogenic Na^+–Ca^{2+} pump which exchanges Na^+ and Ca^{2+} according to the stoichiometry: $1Ca^{2+}$ (out):$3Na^+$ (in), resulting in a net inward charge transfer (Blaustein, 1988). A rise in intracellular Ca^{2+} may lead to the activation of such an electrogenic pump in neocortical neurones (Friedman, Arens, Heinemann & Gutnick, 1992), resulting in a depolarization of the cell membrane with little change in somatic conductance. However, the reversal potential for the exchange current is very depolarized, and the depolarization should have been larger at hyperpolarized potentials. Voltage clamp studies aimed at isolating the Na^+–Ca^{2+} pump or a mixed cationic conductance in VP neurones may resolve this issue.

Based on the data presented here and those of Li & Hatton (1996), the reduction of I_{KL} and a Ca^{2+}- and Na^+-dependent mechanism probably both contribute to the histamine-induced depolarization. The relative importance of each of these processes could depend upon the availability of cytoplasmic Ca^{2+}. Since we found that reducing extracellular Na^+ had more profound effects on the histamine-induced depolarization than did reduction of the driving force for K^+, it seems likely that Na^+ entry is a necessary component of the effect, at least when the intracellular milieu of the cell is not experimentally altered. A combination of the two mechanisms probably accounts for the fact that we and others (Li & Hatton, 1996) could not detect any consistent histamine-induced input resistance changes under normal conditions, especially if the

conductances take place on the dendrites. Alternatively, histaminergic activation of one or the other mechanism might prevail, depending on the metabolic state of the individual neurone.

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